

Acoustic System for Insect Detection in Plant Stems: Comparisons of *Cephus cinctus* in Wheat and *Metamasius callizona* in Bromeliads^{1,2}

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ABSTRACT *Cephus cinctus* Norton (Hymenoptera: Cephidae) larvae feed and pupate inside wheat stems, hidden from predation and behavioral observation. Larval activity can be detected acoustically by attaching an accelerometer to the base of an infested stem. However, the initial procedure used to clamp the accelerometer to a stem was difficult to perform and sometimes compressed weak stems. An improved, nondestructive clamping device was developed and tested in studies that compared sounds produced by *C. cinctus* in wheat stems with sounds produced by much larger, *Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae) larvae hidden inside meristematic tissue of *Tillandsia utriculata* (L.), an endangered species of bromeliad. Temporal and frequency patterns of sound pulses generated by the larvae were compared in relation to insect size and differences in the structural properties of the substrate. Larvae of both species produce brief, >1 kHz sounds, loud enough to be detected by an acoustic sensor in moderate background noise without using an insulated room or anechoic chamber. As expected, the larger (300–400 mg) *M. callizona* generated louder sounds than the 3–8 mg *C. cinctus*. The improved accelerometer/clamp system can be a useful tool for detecting the movement and feeding activity of *C. cinctus*, *M. callizona*, and other insects that are difficult to observe inside plant tissue.

KEY WORDS sound, feeding and movement behavior, hidden infestations

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a pest of wheat in the northern Great Plains (Ainslie 1920, Morrill et al. 2000). *Cephus cinctus* larvae are difficult to study because they feed and remain hidden within individual wheat stems. One potential method for detecting and monitor-

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ing larval activity in the laboratory involves the use of acoustic sensors, a variety of which have been used to detect cryptically feeding pests. Piezoelectric microphones have been used in several studies (e.g., Vick et al. 1988, Mankin et al. 1999) to detect and monitor activity of stored product insects in grain. Ultrasonic sensors have been used to monitor insects in grain (Shade et al. 1990, Pittendrigh et al. 1997) and wood (e.g., Lemaster et al. 1997, Scheffrahn et al. 1997). Standard microphones with resonant-cavity interfaces have been used to detect and monitor insects in fruit (Webb et al. 1988). Phonograph needles (e.g., Stewart 1997) and accelerometers (Mankin et al. 2000a) have been used to detect and monitor insect activity on or inside plants. The successes of these techniques depended in part on the suitability of the interface between the sensor and the substrate containing the insect.

An initial test of whether an accelerometer system could be used to detect *C. cinctus* activity was successful (Mankin et al. 2000a), but it revealed some practical difficulties with the interface between the accelerometer and the wheat stem. To detect vibrations reliably, the accelerometer must be firmly attached or clamped to the substrate (Anonymous 1989). The screw-plate clamp used in the initial *C. cinctus* studies could be firmly attached to the wheat stem, but it was difficult to judge the number of turns that exceeded the stem load capability (Niklas & Spatz 1999). This proved problematic, as one of the goals of the study was to avoid damage and enable long-term studies on activity in individual stems. Additionally, it was difficult to attach the plate low enough on the stem to avoid it bending under the unbalanced weight of the accelerometer. If the stem did bend, it generated clicks that were difficult to distinguish from larval activity (Mankin et al. 2000a). This report describes an improved technique for detecting *C. cinctus* larval behavior inside wheat stems and compares sounds produced by these small, 3–8 mg larvae with sounds produced by larger, 300–400 mg *Metamasius callizona* (Chevrolat) (Coleoptera: Curculionidae) larvae. *M. callizona* is a recently introduced pest in Florida. The larvae burrow into and destroy the meristematic tissue of bromeliads (Frank & Thomas 1991, 1994, Frank 1997). The adults feed on the leaves (Frank & Thomas 1994). Like *C. cinctus*, *M. callizona* larvae are difficult to detect and study because of their cryptic behavior.

Materials and Methods

Insects and infestation procedures. In an initial test of feasibility, small pots of 'McNeal' spring wheat, *Triticum aestivum* L., were infested with *C. cinctus* larvae by placing the pots into a greenhouse oviposition cage and releasing mated females. The wheat was fully headed when acoustic recordings (see below) were made 3 wks later. A single, recording of at least 120 sec was obtained from each stem. A second test was conducted with younger, 8–21-d-old larvae to consider the potential of the system to conduct long-term monitoring of behavioral activity. The infestation procedure was the same as in the initial test except for 2 stems within which an egg was deposited by hand. Recordings were made from these stems and 13 others that had been exposed in the oviposition cage. Control recordings were made from 10 unexposed wheat stems. The laboratory temperature was 20–23°C.

Comparative recordings were made to detect natural infestations of *M. callizona* in an endangered species of bromeliad, *Tillandsia utriculata* (L.). The bro-

meliads were taken from a colony of *M. callizona* in a University of Florida greenhouse. The recordings were conducted in an air-conditioned laboratory at a temperature of 23–26°C with no special acoustic shielding precautions. Altogether, 94 wheat stems and 7 *T. utriculata* were tested individually and then examined to verify the presence or absence of insects.

Acoustic recording and analysis. The acoustic sensors were Brüel and Kjær ([B&K] Nærum, Denmark) accelerometer systems. In the initial feasibility tests, a B&K model 4371 accelerometer weighing 11 g (Mankin et al. 2000a) was attached to the wheat stem with a screw-plate clamp (Fig. 1A). The second set of tests with younger larvae was done with a counterbalanced alligator-clip clamp that had been designed for rapid attachment and reduced stem compression (Fig. 1B). All tests with *M. callizona* were done by attaching a B&K model 4370 accelerometer weighing 54 g to a *T. utriculata* leaf with the alligator-clip clamp.

The accelerometer signals were transmitted to a B&K model 2635 charge amplifier and recorded on a Panasonic (Matsushita Electric, New York) model SV-255 or a TEAC (Montebello, California) model DA-P1 digital audio tape recorder. Afterward, the recorded signals were digitized at 25 kHz and analyzed on a personal computer with the DAVIS insect signal analysis program (Mankin 1994, Mankin et al. 2000a,b).

The analysis process included the identification of short, high-frequency sound pulses (ticks) and the matching of the pulses against spectral profiles. The profiles were averaged spectra of multiple sounds from relatively noise-free sections of recordings where larvae had been confirmed to be present (Mankin et al. 2000a). The profiles used in this study were derived from 3 files recorded from *C. cinctus* of different ages. The 3 profiles were averages of 21, 20, and 8 pulses in relatively noise-free segments recorded from 14-, 17-, and 20-day-old larvae, respectively. A 512-point spectrum and a 3-msec sampling window around the peak of each pulse were used to construct the profiles and to match individual sound pulses with each profile (for more explanation, see Mankin 1994, Mankin et al. 2000a, b).

In the studies for this report, signals at frequencies below 0.3 kHz were digitally filtered to remove contributions from unshielded background noise (see next paragraph). Absolute Vibration Levels were measured as acceleration in dB referenced to a threshold of 10^{-6} ms^{-2} (Beranek & Ver 1992) between the frequencies of 0.3 and 5 kHz (written as dB/0.3–5 kHz). Relative vibration levels were measured in dB relative to the peak Vibration Level (VL) (see Beranek & Ver 1992, Mankin et al. 2000a for further explanation of these terms). Consequently, the scale is positive for the absolute dB values, and negative for the relative dB values. The two scales can be aligned by noting that the peak Vibration Level for a sound made by a sawfly larva was about 76–80 dB on an absolute scale (see Results below).

Because it was expected that future studies would be done without the use of an anechoic chamber or other sound-reduction precautions (e.g., Mankin et al. 1996), the *C. cinctus* sounds were recorded in a laboratory without acoustic shielding. However, sound-producing electrical machinery and air conditioning equipment were turned off. No special noise-reduction precautions were taken during the *M. callizona* recordings.

Analysis of variance of sound rates and insect weight was performed using SAS Proc Anova (SAS Institute 1988).

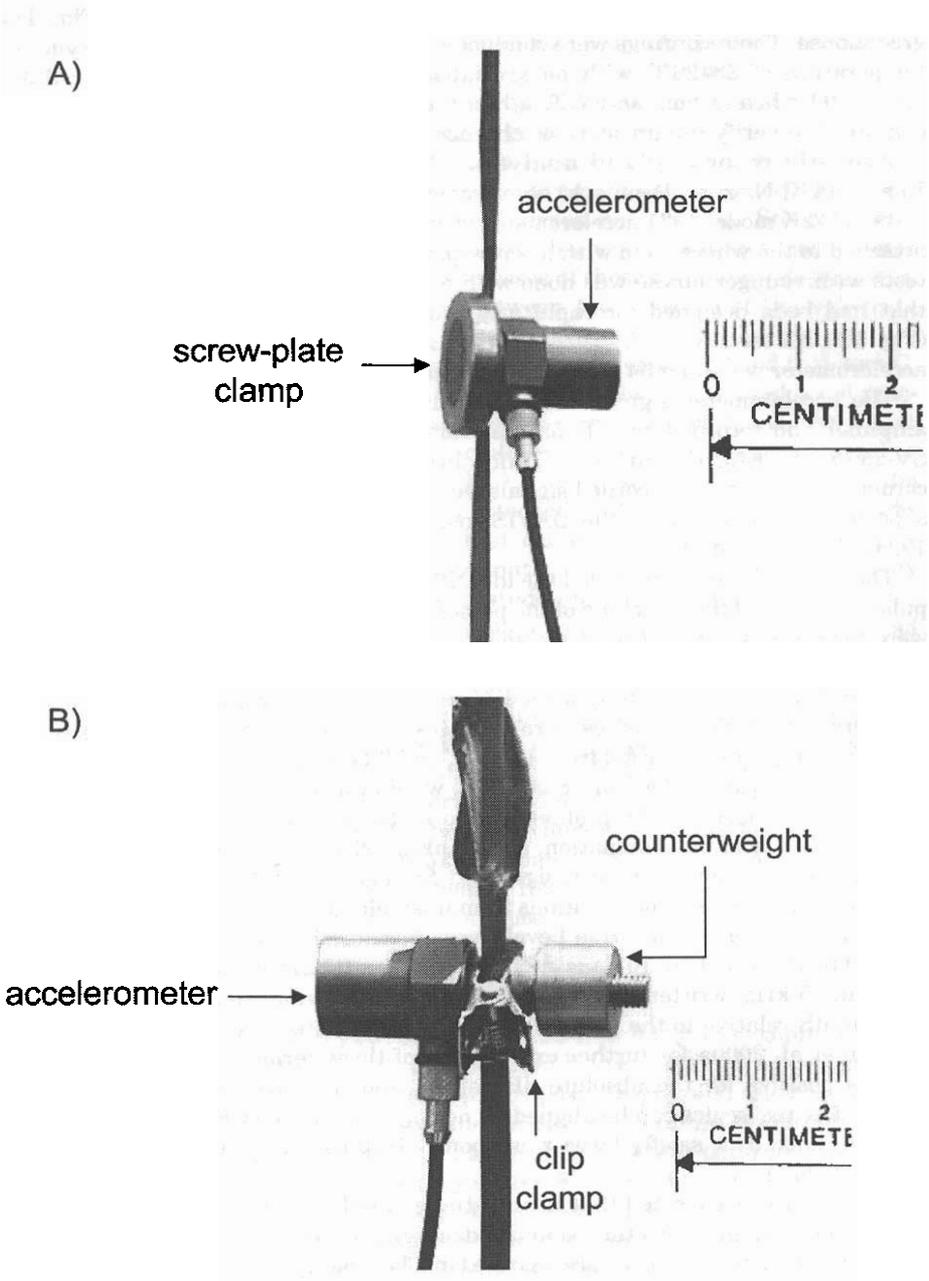


Fig. 1. Accelerometer clamp systems used for detecting insects inside plants. **A)** clamp with screw that attaches directly to accelerometer. **B)** alligator-clip with counterweight to offset bending caused by accelerometer.

Results and Discussion

Sounds from larvae in wheat stems and bromeliad meristematic tissue were detected easily with both accelerometer-attachment systems, with signal to noise ratios of 2:1 or greater (Fig. 2). The oscillogram in Fig. 2A displays sounds made by a *C. cinctus* larva recorded using the model 4371 accelerometer with the screw-plate clamp. The oscillogram in Fig. 2B displays sounds made by a *M. callizona* larva recorded using the model 4370 accelerometer with the alligator-clip clamp. The primary practical difference between the two attachment systems was that the alligator-clip was easier to attach and unhook than the screw-plate, and we observed fewer occurrences of stem-bending because the weight was balanced on both sides of the stem.

***C. cinctus* sound pulse characteristics.** In the initial test of feasibility, sounds were detected in 18 of 42 wheat stems. Individual *C. cinctus* larvae were found in 17 of these stems, and 136 sounds that matched the 3 *C. cinctus* spectral profiles were recorded over a 5,661-sec period. The rate of sounds from unshielded laboratory noises was variable, but typically below 2–3 sounds/min. No *C. cinctus* profile matches were found in 24 stems that contained no larvae. The mean weight of the larvae recovered was 5.25 ± 0.78 mg. There was no significant relationship between the rate of sound production and larval weight ($r^2 = 0.02$, $df = 11$, $P = 0.69$). The numbers of larvae tested and the range of larval weights may have been insufficient to demonstrate an expected correlation (see comparative analysis section below).

In the second test, examining the potential effects of larval age, sounds were detected in 15 infested stems and no sounds were detected in the 10 uninfested stems. The results from the 13 naturally infested and the 2 artificially infested stems were combined because there were no obvious differences in the sounds produced. The averaged sound spectra exhibited a sharp peak near 1.7 kHz and a broader group of peaks between 2.5 kHz and 4.5 kHz (see examples in Fig. 3), similar to the pattern seen previously (Mankin et al. 2000a). There was a somewhat stronger high-frequency component in pulses made by older larvae. Otherwise, there was no discernible effect of age on the sawfly sound-pulse spectra (Fig. 3). The mean rate of sounds was 0.063 ± 0.015 s⁻¹, somewhat higher than the mean of 0.024 ± 0.004 s⁻¹ in the initial study. There was no discernible effect of age on the rate of sound production. The standard errors in these small-scale studies are probably not indicative of the full range of values that would be found in a long-term study.

The mean vibration levels in recordings from *C. cinctus* of different ages (Fig. 4) ranged from near background, 60–65 dB, to moderately low intensities, 81–82 dB, approaching the threshold of human perception (e.g., Parsons & Griffin 1988). In Fig. 4, the signals appear to separate into a group of low-intensity sounds <70 dB, and a group of higher intensity sounds ≥ 73 dB, with no discernible effect of age on vibration level. Although we had expected that older, larger larvae might produce louder sounds than young larvae, there was no discernible effect of age on the sound intensity level.

***M. callizona* sound pulse characteristics.** Sounds were detected in 5 of the 7 *T. utriculata* tested. Each of these contained 1 or more *M. callizona* larvae, pupae, or adults. We did not calculate sound pulse rates from these recordings because it was not always certain which sounds came from which insect, but in

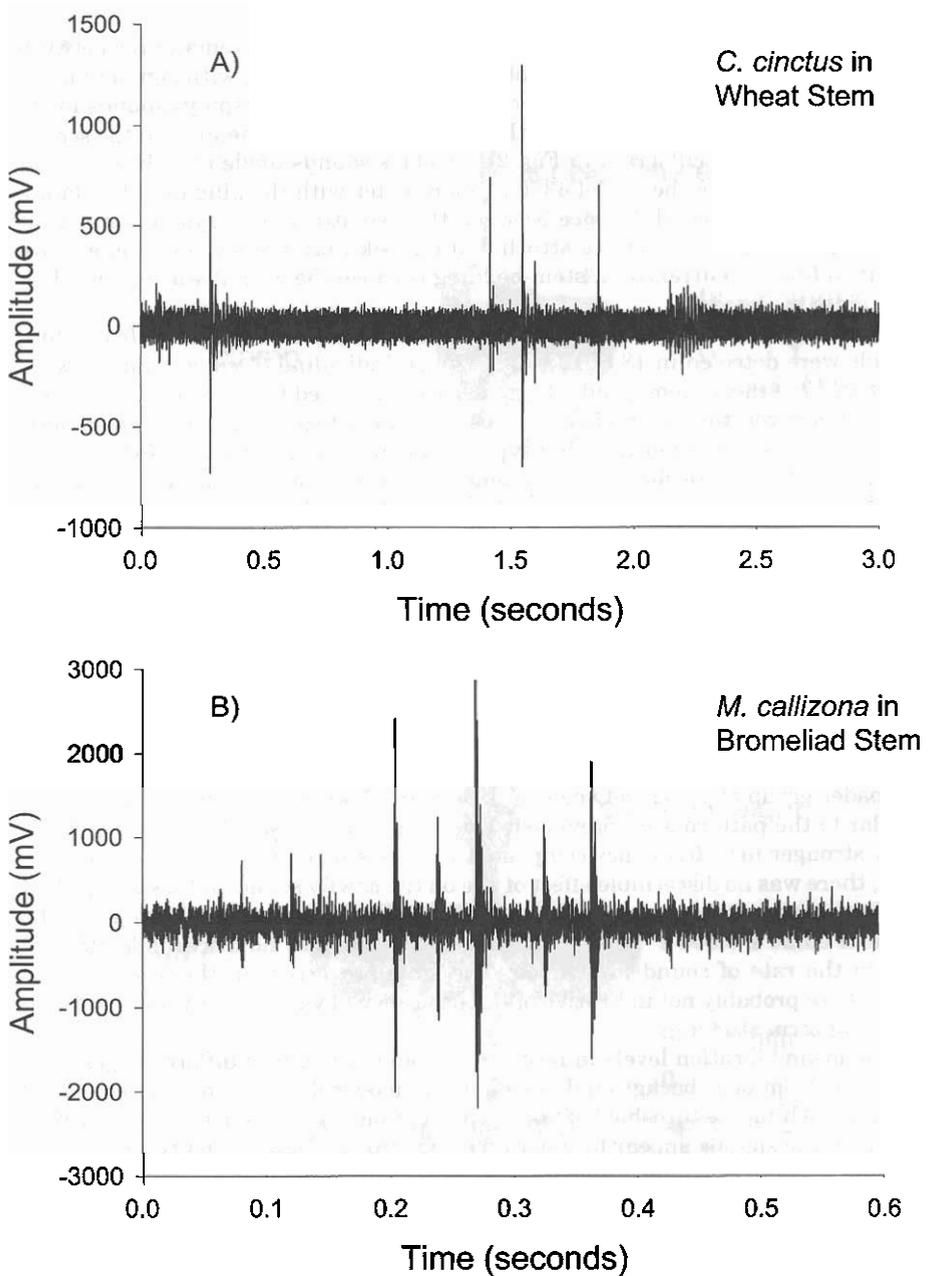


Fig. 2. Oscillograms of larval sounds. A) *C. cinctus* larva inside a wheat stem. B) *M. callizona* larva in bromeliad meristematic tissue.

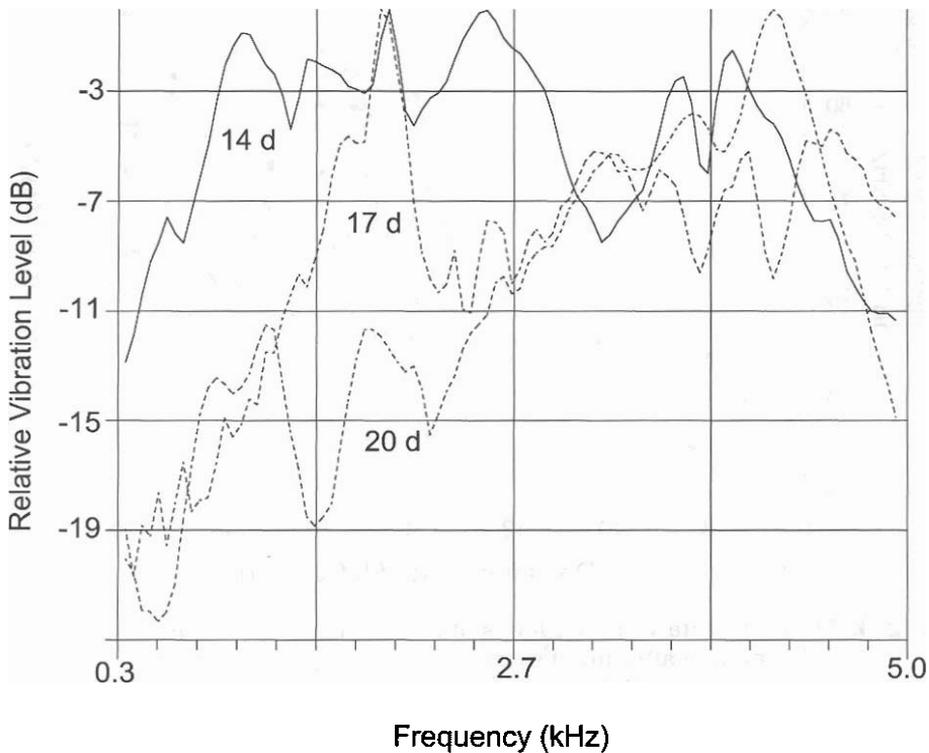


Fig. 3. Mean spectra in recordings of sound pulses made by 14-, 17-, and 20-d-old *C. cinctus* larvae in wheat stems. These spectra were used as profiles to distinguish insect sounds from background noise.

general, the sounds were more easily detectable and more frequent than in the sawfly recordings. The adults produced louder sounds (85 dB) than the larvae (80 dB), with stronger low-frequency components (Fig. 5). One of the 2 *T. urticulata* from which no sounds were detected contained a large larva that apparently was not moving or feeding during the recording period. A small, dead larva was found in the 2nd plant.

Comparative analysis. The differences between the sounds of adult and larval *M. callizona* (Fig. 5), and between *M. callizona* and *C. cinctus* larvae (Figs. 3 and 5) followed a general pattern observed in previous studies (Pittendrigh et al. 1997, Mankin et al. 2000a). Larger insects, when active, tend to produce louder and more frequent sounds than smaller insects because their movements tend to contain more energy. However, the loudness and the rate of sound production usually are not fail-safe indicators of larval size or age because they are highly variable and they decrease during periods of molting or pupation. In the 2 series of *C. cinctus* tests above, the individual sound pulse spectral patterns and the mean vibration levels are too variable for use as reliable indicators of age. As expected, however, the *M. callizona* larvae produced louder sounds than *C. cinctus* larvae (Figs. 4–5).

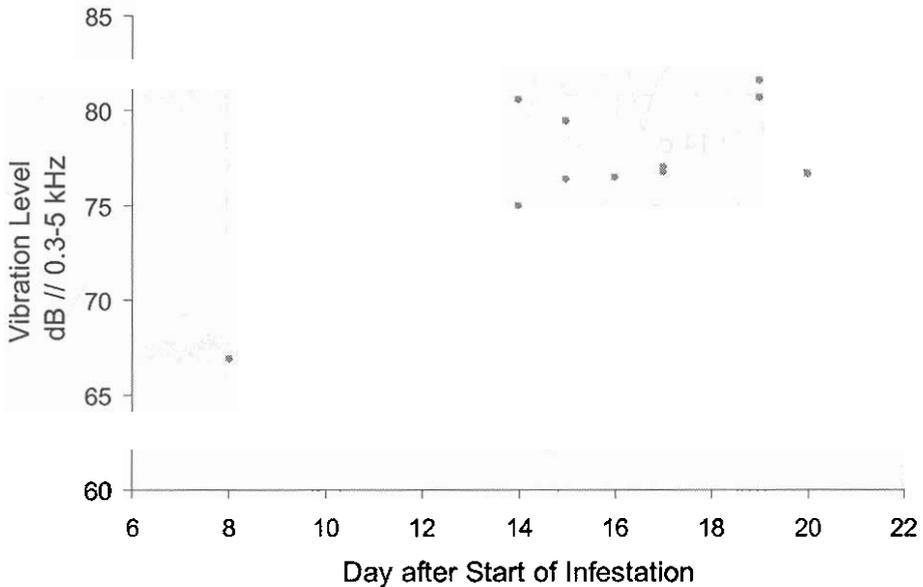


Fig. 4. Mean absolute vibration levels of sounds made by *C. cinctus* larvae at different ages after infestation.

Some of the spectral differences between the *C. cinctus* and *M. callizona* sounds can be attributed to differences between the sound transmission characteristics of wheat stems and bromeliad meristematic tissue. The stem is a nearly ideal transmission medium, enabling weak, high-frequency signals generated by small larvae to be transmitted over relatively long distances, as is the case with ultrasonic sounds produced by termites (e.g., Scheffrahn et al. 1997). Bromeliad meristematic tissue has less stiffness than a wheat stem, but it is still a better transmission medium than sand for high frequencies. Consequently, it is not surprising that the peak frequencies detected in sound pulses recorded from larvae in bromeliad tissue were intermediate between the peak frequencies in wheat stems and in soil (Mankin et al. 2000a).

Assessment of utility. As in previous detection studies (e.g., Shuman et al. 1993, Weaver et al. 1997, Mankin et al. 2000a), the ability to predict an infestation depended partly on the insect activity level and partly on background noise. Typically, active insects can be identified quickly and reliably, but the occurrence of loud background noises can produce false positive identifications, and insects that fail to move during the recording period can produce false negatives. The percentages of false positives and false negatives essentially depend on factors that are largely independent of the acoustic system.

The accelerometer system with the alligator-clip clamp has considerable potential as a laboratory tool for monitoring the larval behavior of *C. cinctus* and other insects that feed inside stems and meristematic tissue. The device is easy to attach and it is possible to sample multiple stems quickly to estimate the level of infestation in a pot or small plot of wheat. Because the alligator-clip clamp is less

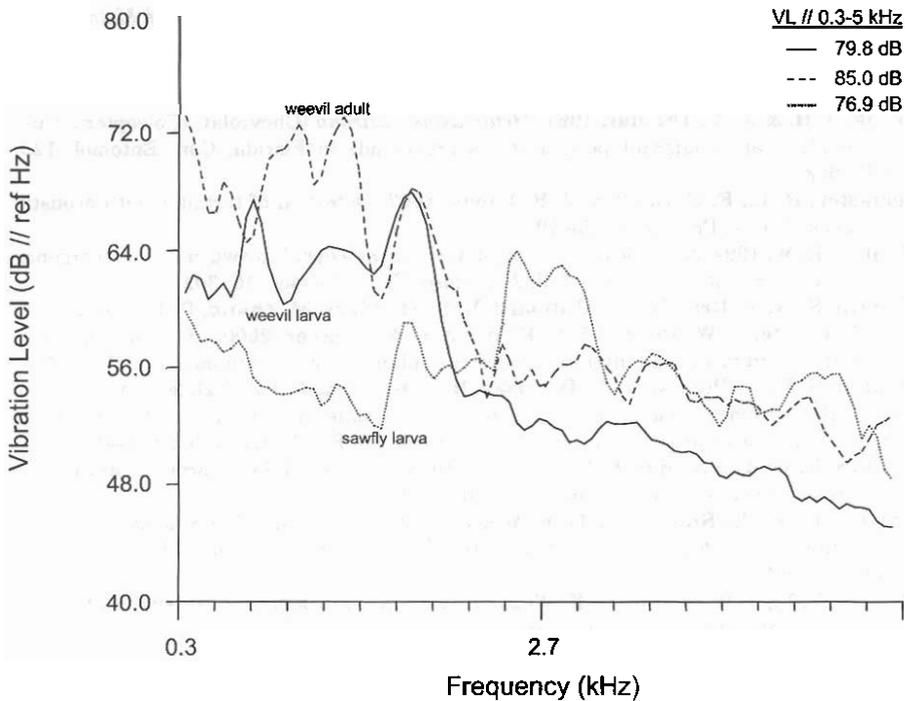


Fig. 5. Comparisons of spectra of *C. cinctus* larval sounds and *M. callizona* larval and adult sounds. Vertical scale indicates absolute vibration level at a specified frequency (dB/ref Hz)

damaging to the stem than the screw-type clamp, it has the potential for multiple reattachment to a single stem or for remaining connected to the stem to monitor long-term activity. The cryptic behavior and small size of *C. cinctus* larvae is no longer a formidable barrier to their behavioral study.

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